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The TBP-Inhibitory Domain of TAF145 Limits the Effects of Nonclassical Transcriptional Activators

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Summary

Many genes in bacteria and eukaryotes are activated by "regulated recruitment" [1]. According to that picture, a transcriptional activator binds cooperatively to DNA with the transcriptional machinery, and the constitutively active polymerase then spontaneously transcribes the gene. An important class of experiments that helped develop this model is called the "activator by-pass" experiment [2–7]. In one version of such an experiment, the ordinary activator-transcriptional machinery interaction is replaced by a heterologous interaction. For example, fusing any of several DNA binding domains to Gal11, a component of the yeast mediator complex [2, 4, 5], creates a powerful activator of genes bearing the corresponding DNA binding sites. Here, we describe a simple modification of the yeast transcriptional machinery that extends the success of similar experiments involving other mediator components. The results reinforce parallels between regulation of enzymes involved in transcription and in other cellular processes.

Results and Discussion

We call proteins bearing a component of the transcriptional machinery fused to a DNA binding domain (e.g., LexA-Gal11) "nonclassical" activators to distinguish them from "classical" activators (e.g., Gal4), which bear natural activating regions. Nonclassical activators are believed to insert into the transcriptional machinery (LexA-Gall11 replacing Gal11 in the mediator, for example), whereas classical activators are believed to touch (with their activating regions) various surfaces on the transcriptional machinery [1].

The experiments reported here were prompted by the puzzling finding that, in yeast, so-called nonclassical activators bearing Gal11 typically work more efficiently than do similar hybrid proteins bearing other mediator components [8]. We wondered whether a modified form of regulated recruitment, illustrated for certain enzymes not involved in transcription, might apply here as well. In those cases, an inhibitory flap helps ensure that the enzyme remains inactive unless complexed with its substrate [9]. For example, the G protein exchange factor SOS bears an inhibitory flap; when that flap is removed, simply tethering the rest of SOS to the membrane suffices for activation of the RAS pathway [10, 11]. We speculated that a corresponding inhibitory flap might be limiting the success of many attempted transcriptional activator by-pass experiments.

Nakatani and colleagues have shown that the transcriptional machinery bears at least one inhibitory flap: TAF145 binds TBP, and its amino domain (called TAND-1) covers the underside of TBP and hinders its binding to DNA [12–14]. Here, we show that removing this inhibitory domain greatly increases the scope and efficiency with which a variety of nonclassical activators work. The flap evidently helps decrease basal expression of genes while having little effect on the action of classical activators.

Figure 1 shows that deletion of TAND-1 from TAF145 greatly increased the activity of several nonclassical activators. These nonclassical activators comprise a mediator component, Gal11, SRB4, SRB6, Med1, or Med6, fused to a zinc finger DNA binding domain called Zif. The reporter gene bears two Zif binding sites (each of which binds a Zif monomer) upstream of a *CYC1::LacZ* fusion in Figure 1A and upstream of a *Gal1::LacZ* fusion in Figure 1B. As assayed with either reporter, the Med and SRB fusions worked about 12- to 20-fold more efficiently in the TAND-1 deleted strains than they did in the wild-type strain. The deletion increased the activity of the Gal11 fusion about 5- to 7-fold and increased that of Gal4-Zif (a classical activator) only about 2-fold. Thus, in the TAND-1 deleted strain, Gal11-Zif worked almost twice as efficiently as did Gal4-Zif. Transcription elicited by Gal4 itself, working from Gal4 sites, was increased less than 2-fold by the TAND-1 deletion (data not shown). We noted in one experiment that the effect of deleting TAND-1 was smaller (but qualitatively the same) when the activator binding sites were moved further upstream (data not shown), but we have not investigated systematically the effect of activator positioning. Removal of TAND-1 had no discernable effect on the level of expression of TAF145 or of the activator Gal11-Zif (data not shown).

Figures 1A and 1B also show that basal transcription (i.e., that observed absent the effect of any known activator) was increased some 3- to 4-fold by deletion of TAND-1, as assayed with either reporter (columns 1 and 2.) A comparison of activated versus basal transcription reveals that the TAND-1 deletion decreased the "fold stimulation" by the classical activator Gal4-Zif with either reporter (some 2-fold) while greatly increasing the fold stimulation by the various nonclassical activators tested.

Figure 2 shows that the activity of a classical activator (Gal4-Zif) and two nonclassical activators (Gal11- and SRB4-Zif), as well as basal transcription, required an intact TATAA whether or not TAND-1 was present.

We repeated these experiments with several nonclassical activators bearing the LexA DNA binding domain instead of the Zif binding domain and providing LexA

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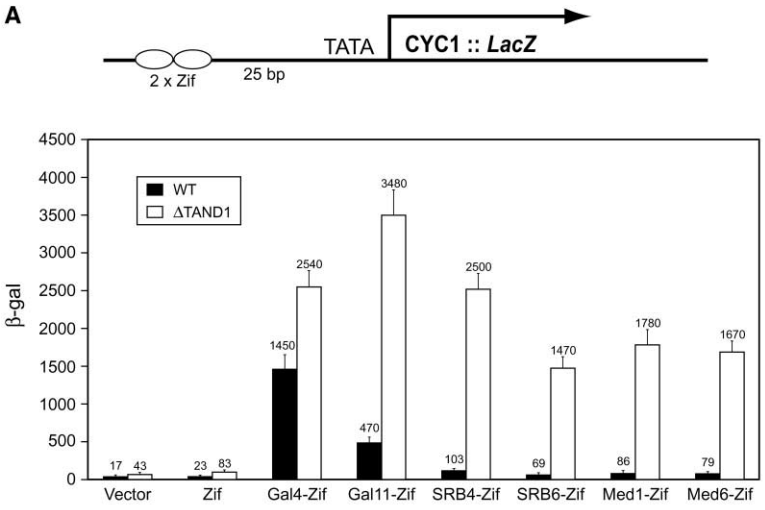

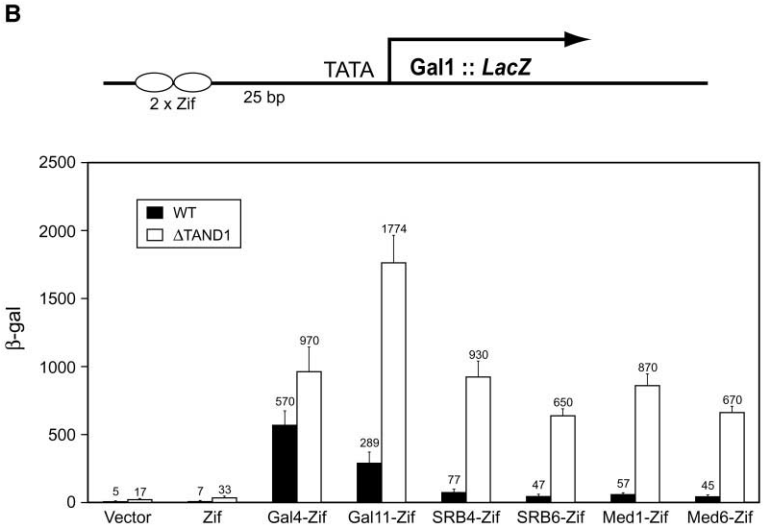

Figure 1. Deletion of TAND-1 Preferentially Increases the Activities of Nonclassical Activators

(A and B) Transcriptional activity elicited by the classical activator Gal4-Zif and by various nonclassical activators (all of which bear the Zif DNA binding domain) in wild-type (WT) and TAND-1-deleted strains. In (A), the reporter is a *CYC1::LacZ* derivative, and, in (B), the reporter is a *Gal1::LacZ* derivative. Reporters used here and throughout this paper were integrated at the *Ura3* locus. Genes encoding the various activators were expressed from the ADH1 promoter and were carried on single-copy plasmids. Data are from at least three independent samples for each column here and in all subsequent figures. "Vector" signifies the presence of the plasmid encoding no relevant function; "Zif" signifies that the plasmid expresses the Zif DNA binding domain unattached to any other protein or peptide.

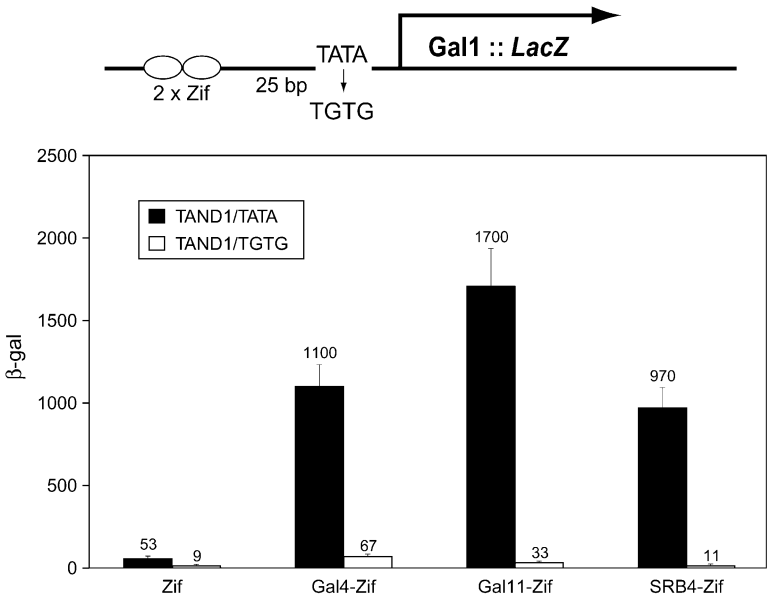

Figure 2. Mutation of TATA Box Abolishes Transcription in TAND-1-Deleted Yeast Strains

The TATA box in the *GAL1* promoter of the *lacZ* reporter was changed as shown.

sites in place of Zif sites in the reporters. We found that the activity of LexA-Gal11, about equal to that of LexA-Gal4 in wild-type strains, was increased some 3-fold by the TAND-1 deletion, and found that the activity of LexA-Gal4 was essentially unchanged. Other nonclassical activators bearing LexA (e.g., LexA-SRB4) elicited little transcription in either strain (data not shown). This lack of activity might be accounted for by the fact that the LexA domain is attached to the amino end of the mediator component in each case (unlike the Zif domain, which is attached at the carboxyl end) or by the fact that the Zif DNA binding domain has a higher affinity for DNA than does the LexA domain. That the latter explanation is correct is suggested by the finding that a triple fusion protein comprising LexA-SRB4-Zif activated a Zif site-containing reporter at high levels in the TAND-1 strain (but not in the wild-type strain), whereas it worked poorly on LexA site-containing reporters in either strain (data not shown).

The experiments in Figures 1 and 2 were performed with strains bearing chromosomal deletions of *TAF145*, and the wild-type and TAND-1 mutant forms of the TAF145 were encoded on low-copy (*ARS/CEN*) plasmids. In other experiments, we found that overproduction of the two forms of TAF145 (carried on multicopy plasmids), in wild-type strains, elicited effects similar to those shown in the figures (data not shown). This is the result expected if the overproduced mutant TAF displaces the wild-type form on TBP.

The experiments of Figures 1A and 1B were also performed with a mutant TAF145 deleted for both TAND-1 and the adjacent domain called TAND-2 (which binds to the top of TBP). In all cases, the results were similar to those described here, but of smaller magnitude (data not shown).

Conclusions

In summary, many of the nonclassical activators we tested worked significantly better in cells bearing a TAF145 deleted for the TBP-inhibitory domain called TAND-1 [12–14]. In such strains, for example, SRB4-Zif worked 12- to 20-fold as efficiently as it did in wild-type cells, and Gal11-Zif worked some 5- to 7-fold more efficiently. Because the activity of Gal4 itself was affected only modestly by the deletion (as were the activities of fusion proteins bearing Gal4's activating region), in such strains, Gal11-Zif worked *more* efficiently than did Gal4, and SRB4-Zif's activity was about equal to that of Gal4. The results show how a simple modification of the transcriptional machinery can greatly extend the success of one class of activator by-pass experiments.

We do not know why deletion of TAND-1 has a particularly significant effect on activation by nonclassical activators. A hint might be found in the suggestion that activation of the *Gal1* gene by Gal4 is TAF145 independent [15–17]. Perhaps Gal4 activates by recruiting a form of TBP that is free of this inhibiting TAF, whereas the nonclassical activators cannot do so. The latter, for example, might recruit the mediator [18] and then depend on spontaneous release of the inhibiting flap for assembly of an active transcription complex. A variant of the idea that at least some classical activators recruit a TAF145-free form of TBP is that such activators might directly open the inhibitory flap on the TAF (see [12] and [19] for details).

Our findings (see [19] and [20] for details) also indicate that the TAND-1 domain helps to maintain a low level of basal transcription but has little effect on transcription elicited by classical activators. In this regard, the TAND-1 serves a function analogous to that of the inhibitory flap (domain) on SOS mentioned above. In each case, the inhibitory domain helps suppress the activity of an enzyme (RNA polymerase, in one case, which requires TBP binding) and SOS (in the other case) in the absence of a specific inducing signal.

Experimental Procedures

We used a yeast strain deleted for TAF145 and carrying a single-copy plasmid expressing either wild-type TAF145 or a TAF145 mutant deleted for residues 10–43 (i.e., a deletion of TAND-1). These strains are also *MAT*α *ura3-52 trp1-63* and *leu2,3-112*. They were derived by Kotani et al. [21] and were kindly provided to us by Dr. T. Kokubo. To construct the reporter plasmids, two copies of the Zif binding site were inserted 25 bp upstream of the TATA box of the *CYC1* and *Gal1* genes. The most upstream three TATA boxes of the *CYC1* promoter were deleted in the *CYC1* promoter-driven LacZ reporter used in this study. All reporters were inserted at the *Ura3* locus. Each 210 amino acid "Zif" domain contains four C2H2 zinc fingers that contact DNA (J.X.C. and R.L. Juliano, unpublished data). This domain binds to DNA as a monomer, each monomer recognizing the sequence 5′-GAGGCGGCGTGGC-3′. Plasmids expressing activators were derived from pXC1 [22]. The hybrid proteins comprise the Zif domain fused at its amino terminus to either Gal4 (residues 147–881) or to the full-length Gal11, SRB4, SRB6, Med1, and Med6 proteins. Assays were performed by growing cells in synthetic medium lacking uracil, tryptophane, leucine, and histidine to maintain the plasmids, and the medium contained 2% galactose and 2% raffinose. Cells were grown at 30°C overnight (to OD_{600} of about 1.0), harvested, and assayed for β-galactosidase activity as described previously [8].

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